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# Factor V Leiden Is Not Responsible for Stroke in Patients With Sickling Disorders and Is Uncommon in African Americans With Sickle Cell Disease

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**Cerebrovascular accidents in patients with sickle cell anemia are among the most devastating complications of the disease. It has recently been demonstrated that some patients have a hypercoagulable state on the basis of the presence of an abnormal factor V molecule, factor V Leiden. We undertook this study to evaluate the presence of factor V Leiden in sickle cell patients with stroke. Eighty-two patients with either Hgb SS, Hgb SC, or Hgb S$\beta^+$-thalassemia comprised the study population. Of the 82 patients in the study, 19 of them had a history of stroke. In our study population, none of the stroke patients possessed the factor V Leiden mutation. One of the non-stroke patients was a heterozygote for the mutation (*P* = 1.00). The overall frequency of the factor V Leiden allele in our population is 0.6%. The estimated prevalence for this mutation is reportedly between 3 and 7% in Caucasian populations. We conclude that the gene frequency for factor V Leiden is less common in Africa Americans with sickle cell disease. Furthermore, factor V Leiden does not appear to be responsible for the development of stroke in sickle cell patients. Am. J. Hematol. 54:12–15, 1997.** 

**Key words: thrombosis; hemoglobinopathy; PCR**

## INTRODUCTION

Activated protein C (APC) is a serine protease that is formed from an inactive precursor when protein C interacts with thrombin bound to the endothelial receptor thrombomodulin.[1,2] Activated protein C in turn inactivates factors Va and VIIIa by enzymatic cleavage.[3] Recent studies have suggested that the most frequently identified laboratory abnormality in patients with venous thrombosis is resistance to activated protein C.[4] In fact, up to 64% of patients with thrombophilia exhibit resistance to activated protein C.[5] It has recently been shown that one etiology for activated protein C resistance is heterozygosity or homozygosity for a single point mutation in the factor V gene, which leads to an abnormal factor V molecule (factor V Leiden) that is not properly inactivated by activated protein C.[6,7] This mutation at nucleotide position 1,691 is a G to A transition that replaces arginine at position 506 with glutamine. It produces a factor V that is resistant to proteolysis by activated protein C. The estimated prevalence of this mutation is reported to be between 3 and 7%.

Cerebrovascular accidents in patients with sickle cell anemia are among the most devastating complications of this disease. Clinically apparent strokes occur with an estimated frequency of up to 10%, with a recurrence rate for subsequent strokes of 70%.[8] The peak age of incidence

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for stroke in sickle cell patients is between the ages of 1 and 15 years.[9] Stroke, in patients with sickling disorders, can be either thrombotic or hemorrhagic, with the majority of strokes in sickle cell patients being thrombotic.[10] Previously identified risk factors for stroke in sickle cell patients include the presence of Hgb SS, high leukocyte counts, higher steady-state Hgb A2 levels, lower hemoglobins, higher reticulocyte counts, and the presence of cardiomegaly.

Because the incidence of factor V Leiden in control populations approximates the incidence of stroke in the sickle cell population, we undertook this study to determine if factor V Leiden is found with increased frequency in sickle cell patients with stroke. We also performed this study to estimate the frequency of the factor V Leiden gene in an African American population with sickle cell disease, a group that to our knowledge, has not been studied previously.

## MATERIALS AND METHODS

Eighty-two patients with Hgb SS, Hgb SC, or Hgb S$\beta^+$-thalassemia who were followed in our sickle cell center were entered onto the study. Informed consent was received from each patient. The study was approved by the institutional review board and human subjects committees at our institution. Following study entry, 10 ml of EDTA anti-coagulated blood was drawn from the antecubital fossa of each patient. Blood was sent for analysis at room temperature. The samples were not identified as to history of stroke. Of the 82 patients, 19 had a documented history of stroke.

Centrifugation was used to separate a mononuclear cell fraction. From this cellular fraction, DNA was prepared from the peripheral blood leukocytes by standard techniques.[11] Similar to other reports, the region of exon 10 of interest was amplified from genomic DNA with two primers—5′—GGG CTA ATA GGA CTA CTT CTA ATC—3′ and 5′—TCT CTT GAA GGA AAT GCC CCA TTA—3′. The polymerase chain reaction (PCR) conditions were 5 min of initial denaturation at 94°C followed by 30 cycles of 60 sec denaturation at 93°C, 30 sec annealing at 61°C, and 90 sec extension at 72°C.[12] The 161 bp amplified product was digested with the restriction enzyme *Mnl*I. This produces two fragments of 43 and 118 bp. AG to A mutation at nucleotide 1,691 in the codon for Arg 506 results in a loss of this cleavage site. The subsequent cleavage products were therefore run on an agarose gel containing ethidium bromide and analyzed for the presence of factor V Leiden.

Sickle cell patients with and without stroke were stratified by presence or absence of factor V Leiden using a $2 \times 2$ contingency table, and the Fisher's exact test was performed to test for a possible relationship. Comparisons between the population of patients with and without stroke were compared using the difference between proportions. Confidence intervals for the allelic frequency of this matation were calculated to compare the prevalence of the mutation in our study with the prevalence previously reported in the literature. *P* values reported are two-tailed.

**TABLE I. Characteristics of Sickle Cell Patients With and Without Stroke**

| | Control | Patients with stroke | *P* value |
|---|---|---|---|
| No. of patients | 63 | 19 | NA |
| Age | 7.5 ± 1.0 | 7.1 ± 1.6 | 0.02 |
| No. with SS | 52 | 19 | 0.05 |
| No. with SC | 9 | 0 | 0.08 |
| No. with S$\beta^+$-thal | 2 | 0 | 0.43 |
| Male/female | 33:30 | 12:7 | 0.44 |

## RESULTS

The clinical characteristics of our patients are shown in Table I. Nineteen of the 82 patients had a history of stroke. The mean age of our study population was 10.5 years (range, 48 days–31 years). There were 45 male and 37 female patients. The patients with stroke were younger than those without a history of stroke. The male to female ratio was similar for the two groups. All the patients with stroke were homozygous for hemoglobin S. In the non-stroke patients, 9 patients had the SC genotype, 2 patients had the S$\beta^+$-thalassemia genotype, and the remaining 52 patients were homozygous for hemoglobin S.

In our study, only one patient was found to be heterozygous for factor V Leiden. This patient had no history of stroke, priapism, or other thrombotic disorders and was homozygous for hemoglobin S. The restriction enzyme analyses for this patient as well as for patients with a normal factor V genotype are shown in Figure 1. We conclude that there is no statistically significant association between the presence of factor V Leiden and past history of stroke in patients with sickle cell disease (Fisher's exact test, $P = 1.0$). To ensure that our detection methods were valid, samples from patients with known genotypes were analyzed in a blinded fashion. We were able to identify the genotypes correctly in all blinded samples.

The allelic frequency for factor V Leiden in this study population was found to be 1 mutation in 164 alleles, or 0.6%, which is significantly different from the lowest prevalence (2.9%) previously reported in the literature (95% confidence interval, 0–2.83%).

## DISCUSSION

This study was performed to see if factor V Leiden was found with increased incidence in patients with sickle

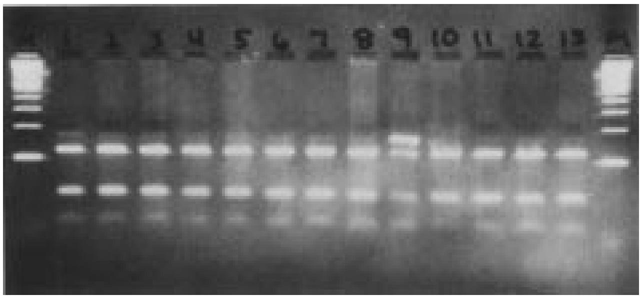

**Fig. 1. Agarose gel electrophoresis for factor V Leiden detection following PCR amplification of DNA from sickle cell patients following digestion with the restriction enzyme *Mnl*I. Normally two fragments of 43 and 118 bp are produced for the wild type (lanes 1–8; 10–13). Lane 9 shows a 161 pb fragment (arrow) in addition to 43 and 118 bp fragments indicating a heterozygous mutant for factor V Leiden. Note that this was not a patient with a history of stroke.**

cell disease. We have demonstrated in our study population that factor V Leiden is not responsible for stroke in patients with sickle cell disease and that this mutation is uncommon in African Americans with sickle cell disease.

The pathogenesis of thrombotic stroke in sickle cell disease is presumed to be secondary to endothelial cell damage occurring in the setting of high cerebral arterial flow rates resulting from a decreased oxygen-carrying capacity of sickled red cells and compensatory arterial dilation.[10] Endothelial damage likely leads to loss of the normal anticoagulant properties present in the endothelium, which in turn leads to thrombus generation.[13] Thrombosis is more likely in areas of turbulence, as is seen distal to an area of obstruction caused by sickled erythrocytes. Normally, fibrin generation is partially inhibited by the protein C pathway. For this reason we investigated whether factor V Leiden, a mutation leading to resistance to activated protein C, may be responsible for stroke in patients with sickle cell disease. In our population, this did not appear to be the case.

Whether factor V Leiden is a risk factor for arterial thrombosis is somewhat controversial. Resistance to activated protein C has been described previously in patients with arterial thrombosis and stroke.[14,15] However, it is unclear in these studies whether resistance to activated protein C was on the basis of factor V Leiden or due to other abnormalities. In a later study of healthy men, factor V Leiden was not associated with an increased risk of either myocardial disease or stroke.[16] In both of these studies an increased incidence in venous thrombosis was noted in the study populations. It therefore appears that the presence of factor V Leiden is more a risk for venous thrombosis than for thrombosis of high flow arterial systems. This also appears to be the case in patients with sickle cell disease.

The estimated frequency of the factor V Leiden maturation ranges from 3 to 7% in control populations.[17–23] In patients with thrombosis, the incidence of this mutation is usually quoted as being five- to tenfold higher. We have found a much lower incidence of this mutation in our study population. Our reported incidence of this mutation in African American patients is similar to another recently published report comparing 214 African American and 126 Caucasian patients in which 1.4% of the African American patients and 1.6% of the Caucasian patients were heterozygous for the factor V Leiden gene.[24] The authors concluded that there was no difference in the incidence of this mutation in the African American and Caucasian populations studied. We comment that the reported prevalence of factor V Leiden in Caucasian patients in this study was lower than that in the reported literature.

If factor V Leiden is found less commonly in African American patients, then it may follow that the incidence of thrombosis would be less in this racial group. Support for this claim comes from a large cohort study involving a random sample of 5% of all Medicare claims made between 1986 and 1989, in which the relative risk of deep venous thrombosis in African American patients was less than that of Caucasian patients.[25] Although this study indicated that the relative risk of pulmonary embolism was higher in African Americans, we argue that this may relate more to clot mobility and less to clot formation.

It is very unlikely that the presence of a sickle hemoglobin gene affects the presence of a mutant factor V gene, as the β-globin gene is on chromosome 11, whereas the factor V gene is found on chromosome 1.[26] It is also unlikely that factor V Leiden is a lethal mutation in patients with sickle cell disease. In the state of Louisiana, it has been estimated, based on gene prevalence, that there should be between 75 and 80 newborns with sickle cell disease born each year; the actual number of sickle cell newborns born in Louisiana was 74 in 1995 (C. Meyers, Genetic Diseases Program, Louisiana office of Public Health, personal communication). This data makes in utero death an unlikely explanation for the low prevalence of factor V Leiden in our sickle cell population. We conclude that factor V Leiden is not responsible for stroke in sickle cell patients and appears to be present at a lower frequency in this population than in others studied.

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